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MicroRNAs in hematopoietic stem cell aging

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ABSTRACT

The functional decline that is observed in HSCs upon aging is attributed mainly to cell intrinsic factors that regulate quiescence, self-renewal and differentiation. MicroRNAs (miRs) have an indispensable role in the regulation of HSCs and have been shown to also regulate processes related to tissue aging in specific cell types. Here we discuss the role of miRs in the regulation of HSC self-renewal and differentiation throughout life and its implications for future research.

1. Introduction

Aging and its detrimental effects on many physiological processes has become a major topic in biomedical research, as life expectancy of the human population is continuously increasing. Important hallmarks of aging encompass a variety of genetic pathways and biochemical processes, including genomic instability, cellular senescence and stem cell exhaustion (López-Otín et al., 2013). The latter is of particular interest in hematology, where the functional decline of aged stem cells manifests in poor tissue function and disease. Hematopoietic stem cells (HSCs) are responsible for the replenishment of all mature blood cell types throughout the entire lifespan of an individual. The maintenance of balance between self-renewal and differentiation of HSCs is tightly regulated but poorly understood. Upon aging, HSCs gradually lose their regenerative potential and show defects when their repopulating potential is tested in transplantation assays. Counterintuitively, whereas the clonal complexity of the hematopoietic system decreases (i.e. blood cell production is derived from fewer and fewer stem cells), at the same time the number of phenotypically defined HCSs increases (Beerman et al., 2010; Ganuza et al., 2019; Pang et al., 2011). Thus, although there are more stem cells, their active contribution to blood cell production decreases. Although age-related changes in the bone marrow microenvironment have been described, the origin of this functional decline of HSCs is attributed mainly to cell intrinsic factors that regulate quiescence, self-renewal and differentiation (De Haan and Lazare, 2018).

2. MicroRNAs as regulatory components in aging HSCs

Intrinsic control of HSC functioning is executed at multiple levels. Firstly, the regulation of gene transcription by transcription factors, guided by epigenetic chromatin and DNA modifications, greatly influences the expression of key stem cell genes. Secondly, after mRNA is transcribed from the genome, multiple factors control the translation of the mRNA into protein. MicroRNAs (miRs) are one of the factors that interfere with gene expression at the mRNA level. As several miRs have been found to play an important role in HSC regulation they are interesting candidates to further study in HSC aging.

2.1. MicroRNAs as gene regulatory agents

MiRs are small non-coding RNAs that regulate gene expression mainly by targeting the 3′ untranslated region (UTR) of mRNAs present in the cytosol. The primary transcript of a miR can originate from an isolated miR gene or from the intronic region of another (host) gene's transcript. After processing by Dicer and Drosha a mature miR remains, which is loaded into the RNA-induced silencing complex (RISC). Mature miRs are typically ~21 base pairs long and one miR often has many different downstream mRNA targets. Physical interaction between a miR and its mRNA targets induces cleavage of the mRNA or blocks the translation of the mRNA into protein (Bartel, 2009; Ha and Kim, 2014). In this way, miRs are able to fine-tune the abundance of different cellular proteins. (Fig. 1)

2.2. MicroRNAs as hematopoietic regulators

Soon after their discovery in *C. elegans* it became clear that miRs have an indispensable role in regulation of development and stem cells (Lee et al., 1993). Where some miRs are general regulators of development, like let-7 in *C. elegans*, other miRs have shown to have highly

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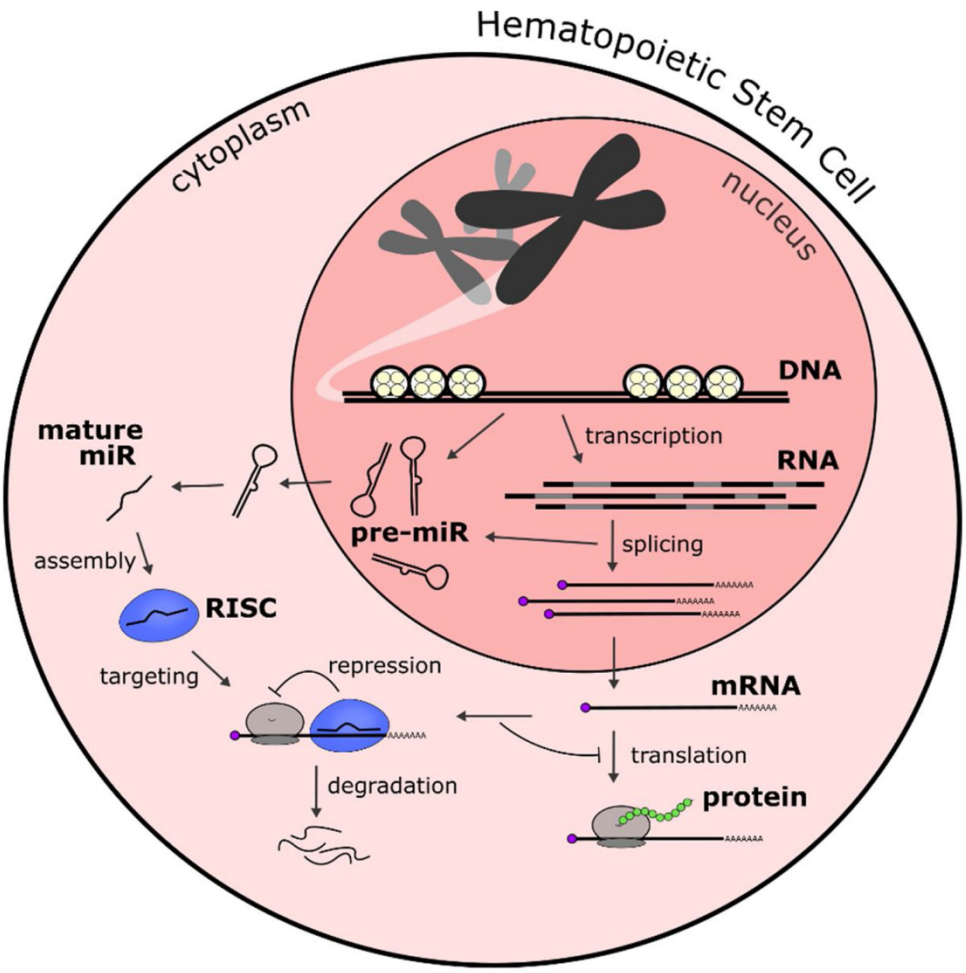

Fig. 1. Intrinsic regulation of HSC gene expression by miRs.
Intrinsic regulation of HSCs takes place at different levels. The regulation of gene transcription by epigenetic modifications and transcription factors controls the expression of different genes. After mRNA is transcribed, miRs can alter protein abundance by inhibiting the translation of their target mRNA into protein by translational repression or endonucleolytic cleavage which results in degradation. Precursor miRs (pre-miR) are translated from their own gene or spliced from an intronic region of another gene. After maturation, the miR is loaded into the RNA-induced silencing complex (RISC) which mediates the interaction between miR and target mRNA.

tissue-specific effects. In the mammalian hematopoietic system, multiple distinct miRs have been found to have specific effects on the behavior of stem cells upon perturbation of their expression. While some miRs, such as miR-181, miR-223 and miR-142 s induce differentiation of HSCs (Chen et al., 2004), the opposite is true for miR-23a, as deletion of this gene results in increased differentiation, suggesting that this miR blocks differentiation. However, when deletion of miR-23a is combined with deletion of the closely related miR-23b, this leads to an overall reduction in hematopoietic stem and progenitor cells (HSPCs) (Kurkewich et al., 2018). These examples, combined with the fact that miRs have many different mRNA targets, show the intricacy of miR networks that regulate HSCs.

2.3. MicroRNAs that regulate quiescence and self-renewal of HSCs

MiRs form intricate networks that regulate many processes in diverse tissues. In HSCs, a limited number of miRs has been found to be specifically associated with maintenance of their quiescence or expansion by targeting various different mRNAs and pathways. Several key examples are listed in Table 1. These miRs are of main interest because they actively stimulate the expansion of HSCs whilst at the same time maintaining their unique stem cell properties. These miRs may therefore be candidates to further investigate their potential use as an HSC expansion- or function-restoring agent. A particularly interesting example is miR-132, which directly targets FOXO3, buffering the increasing expression of this aging related transcription factor and keeping HSCs in a more quiescent state.

2.4. MicroRNAs in aging and age-related hematopoietic dysfunction

Advanced age is associated with a progressive functional decline in many, if not all, tissues. The transcriptome of cells in an aged tissue changes when compared to their younger counterparts, suggesting a role for miRs. Indeed, many reviews highlight the role of miRs in diverse age-associated processes and diseases, such as senescence, Alzheimer's disease, metabolic changes and the regulation of epigenetic changes (Danka Mohammed et al., 2017; Iswariya et al., 2019; Reddy et al., 2017; Victoria et al., 2017). For example, in Alzheimer's disease, it has recently been shown that miR-298 and miR-455−3p protect against abnormal amyloid precursor protein processing by targeting the 3′UTR of the mRNA of this protein (Chopra et al., 2020; Kumar et al., 2019).

There is a plethora of miRs that are associated with many different cancer types through various pathways. These miRs are also referred to as oncomiRs. In the aging hematopoietic system, the risk of leukemia increases with age. In these age-related leukemias many studies have identified miRs that are preferentially expressed in leukemic (stem) cells, suggesting that they contribute to disease. A particularly interesting example is miR-146b, which targets the NF-κB pathway. Deletion of this miR leads to the sustained activation of this pathway and results in the development of hematopoietic malignancy upon aging in mice (Mitsumura et al., 2018).

MiR-29 and miR-101 are miRs that can regulate epigenetic changes by targeting proteins that are part of the epigenetic machinery, such as DNA methyltransferases 3A and B or the Polycomb Repressive Complex 2 protein EZH2 (Amodio et al., 2015; Cho et al., 2011). MiR-29a targeting of DNMT3A induces the maintenance of HSC self-renewal (Hu

Table 1
Examples of miRs that are specifically associated with maintenance of quiescence or expansion through various different targets and pathways.

microRNA	Effect on HSCs	Target	Pathway	Species*
21	Maintains HSC homeostasis (Hu et al., 2020)	PDCD4	Inflammation	M
22	Regulates HSC maintenance and self-renewal (Song et al., 2013)	TET2	Epigenetic	M
29a	Maintains self-renewal (Hu et al., 2015)	DNMT3A	Epigenetic	M
99	Inhibits differentiation and cell cycle entry (Khalaj et al., 2017)	HOXA1	Development	H
125a/b	Induces long-term repopulating abilities in progenitors (Wojtowicz et al., 2016)	MAPK14 (P38)	Inflammation	M/H
126	Drives quiescence and self-renewal in leukemic stem cells (Raffel and Trumpp, 2016)	CDK3	Cell cycle	H
127 − 3p	Limits differentiation (Crisafulli et al., 2019)	*NA*		M
132/212	Regulates HSC maintenance and survival with age (Mehta et al., 2015)	FOXO3	Transcription factor	M
139 − 5p	Regulates proliferation in early hematopoiesis (Choi et al., 2016)	BRG1	Epigenetic	M/H
143/145	Depletion of HSCs, activation of progenitors(Lam et al., 2018)	TGFβ	Growth factor	M
155	Promotes G-CSF induced mobilization (Itkin et al., 2017)	CXCL12	Growth factor	M
193b	Controls HSPC expansion (Haetscher et al., 2015)	c-KIT	Receptor	M
382 − 5p	Supports expansion of granulocyte lineage (Zini et al., 2016)	MXD1	Transcription factor	H

* Species: M = Mus musculus; H = Homo sapiens.

et al., 2015). Interestingly, mutations in DNMT3A are strongly associated with age-related clonal hematopoiesis, and are considered to lead to the emergence of a preleukemic stem cell clone (Ley et al., 2010). Clonal hematopoiesis arises from the preferred outgrowth of an HSC which has acquired a somatic mutation in one of a very restricted number of genes that often encode for proteins involved in epigenetic regulation. A single mutant HSC can give rise to a disproportional amount of progeny, and this phenomenon is strongly age-dependent and occurs in a significant fraction of healthy elderly individuals. Interestingly, some of the targets listed in Table 1, DNMT3A and TET2, are also associated with clonal hematopoiesis (reviewed in: Jaiswal and Ebert, 2019). The epigenetic regulators DNMT3A and TET2 are not only highly prevalent mutations in clonal hematopoiesis, but also influence inflammatory responses of mature cells derived from the mutated HSCs. This indicates that miRs also may have the potential to influence a specific aging phenotype such as clonal hematopoiesis.

3. MicroRNA-based interventions in aging HSCs

Old HSCs display aberrant quiescence, self-renewal and differentiation. MiRs that are able to regulate these processes therefore might be able to restore the homeostasis to the 'young' condition. In this way, miR interventions could potentially be used as a tool to rejuvenate HSCs. Examples of anti-aging interventions that come to mind include the mTOR inhibitor rapamycin and caloric restriction. For the latter there is no evidence that dietary interventions prevent HSCs aging (Lazare et al., 2017). Rapamycin on the other hand might have beneficial effects on aging HSCs (Luo et al., 2014a, 2014b). Rapamycin inhibits the serine/threonine protein kinase mTOR, a regulator of cell growth, metabolism and autophagy. From the miRs highlighted in Table 1, miR-21, miR-22, miR-99, miR-125a/b and miR-155 are all found to target genes in the mTOR pathway (Wang et al., 2018).

3.1. MicroRNAs as rejuvenation tools

In cardiomyocytes it has been shown that the intra-cardiac injection of miR-19a/19b mimics enhances cardiomyocyte proliferation and stimulates cardiac regeneration in response to myocardial infarction injury (Gao et al., 2019). In HSCs, miR-125a may qualify as a HSC rejuvenating factor. Ectopic expression of miR-125a in HSCs results in very potent stem cells with an extended lifespan of individual clones. Moreover, expression of miR-125a in progenitors reverts these cells to a stem cell like phenotype and enables these progenitors to self-renew (a characteristic that they normally lack) and to constitute long-term engraftment. This phenotype was first discovered in murine cells, but is highly evolutionary conserved and confirmed in human cord blood derived HSCs (Wojtowicz et al., 2019). Potentially, the addition of miR-125a mimics to an HSC in vitro culture or even local administration in vivo, could enhance HSC function and functionally rejuvenate old cells by repressing key target genes.

3.2. Target identification and manipulation

Another approach to improve HSCs function may be the direct inhibition of essential miR targets. A single miR has many different mRNA targets, which are typically part of interlinked pathways of proteins or other non-coding RNAs. It is therefore possible to directly inhibit the protein downstream of the miR, as opposed to mimicking the miR itself. This may circumvent some of the issues that come with miR mimics as discussed below. Central to this approach is the notion that targets of candidate miRs are identified, which has turned out to be difficult. Different databases exist that use specific algorithms to predict miR targets. An example is MirTarget, which predicts targets based on the analysis of existing data of miR-target interactions from sequencing experiments (Chen and Wang, 2020; Liu and Wang, 2019). These tools are very useful; however, they often do not provide experimentally validated or tissue specific targets. Different experimental techniques are established for the discovery of indirect and direct targets of miRs, each with their own advantages and caveats (Thomson et al., 2011). Once the regulatory network linked to a miR of interest is pinpointed, this will open up possibilities for direct inhibition of miR targets and mimicking the positive effects that miRs can have on aging HSCs.

4. Future perspectives

Just as a single aging gene does not exist, there is also not a single microRNA that by itself is responsible for the changes observed upon aging. Rather, it may be informative to search for an overall tissue-specific change in miR-profiles associated with aging. Some of these miRs will play a role in more general processes that are shared between different tissues, and some miRs will be linked to specific tissue properties. In the case of HSC aging, differentially expressed miRs will most likely be miRs that are directly linked to stem cell functioning such as self-renewal and differentiation.

Multiple mechanisms are responsible for the functional changes that are observed in the hematopoietic system upon aging. As miRs have been shown to play important roles in HSC self-renewal, it seems likely that miRs are responsible for at least part of the aging phenotype in HSCs. At present, however, our understanding of miR-regulated HSC aging remains poor. MiRs that specifically regulate HSC quiescence and self-renewal would be interesting candidates to further explore their potential as a rejuvenation tool. In order to identify potential deregulated single miRs or miR networks an effort should be made to systematically screen for these changes in young and old hematopoietic stem cells. MiR-knock out (KO) models that allow for conditional KO of miRs can be a valuable resource (Park et al., 2012).

Interference in miR activity could be achieved by mimicking the effects of a miR using RNAi based techniques, however these still come with technical limitations that need to be resolved in the future. In vivo use of miR mimics is hampered by the stability and delivery of these therapeutics (Badalian-Very and Hydbring, 2013). Another issue that will need to be resolved is the activation of the immune system that is inherently linked to the utilization of the RNAi mechanism, which is indispensable for the cell's defense against invading dsRNA and abnormal DNA. Effectively identifying miR regulatory networks in aging cells and tissues, by characterizing and validating mRNA targets, may offer opportunities to manipulate cells, both in vitro and in vivo.

Declaration of Competing Interest

No financial interest/relationships with financial interest relating to the topic of this article have been declared.

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